Fig. 19.—Single specimen after treatment with 2½-per-cent. commercial formalin, showing both the parietal and the more internal portions of the protoplasmic network. The granules are shown only in the upper parietal portion of the network.

Fig. 20.—One extremity of an individual after treatment for 14 days with acidulated pepsin-glycerin. Only the granules of the surface network are represented; these stand out very clearly, but the protoplasmic network itself has been for the most part digested.

Fig. 21.—One extremity of an individual after treatment for 14 days with a concentrated solution of Na_2CO_3. Only the surface network is represented. The protoplasmic network remains clear and distinct, but most of the granules have been dissolved.

The Development of Trypanosoma gambiense *in* Glossina palpalis.

By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captain A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908.)

(Received July 5, 1909.)

[Plates 10 and 11.]

The following experiment is so complete in itself that no apology is offered for publishing it by itself. In 1903 the Sleeping Sickness Commission of the Royal Society came to the conclusion that the carrying of infection from a sleeping sickness patient to a healthy person by the *Glossina palpalis* was a mechanical act, and required no previous development of the parasite within the fly. The Commission also held that the power of transferring the disease was lost to the fly 48 hours after it had fed on an infected person.

Koch and Stuhlmann, in German East Africa, described developing forms in *Glossina*, but did not succeed in infecting healthy animals by the injection of these forms.

Kleine, in German East Africa, at the end of 1908, succeeded first in showing that *Glossina palpalis* could convey *Trypanosoma brucei* some 50 days after the fly had fed on an infected animal.

It seems, at first, strange that this fact should have escaped notice for 15 years, and can only be accounted for by assuming that it is an event of the rarest for a fly to be found which fulfils the unknown conditions necessary for the development of the trypanosomes in its interior. If we assume that it is only one fly in a hundred or in a thousand in which this

development takes place, then the difficulty of observing the phenomenon can be understood.

Take the following experiments, for example:—

Table I.—Flies caught in an Infected Area, kept for some days, and then fed on Healthy Animals.

Trypanosoma brucei—Glossina morsitans.

Expt.	Place.	Observer.	No. of flies fed.	No. of times flies fed.	No. of days before infection or under observation.	Result.
210	Zululand	Bruce	5	32	64	Negative.
242	,,	,,	30	11	56	,,
232A	,,	,,	50	15	34	,,

These experiments seemed to show that if flies caught in a highly infected district, into which a horse could not be taken even for a few hours without contracting nagana, are kept without food for a few days—say three to five—they are then incapable of conveying infection. This appeared to be a strong proof that the duration of infectivity in the fly was a short one, since, if this were not the case, 1 of the 85 flies ought to have been in a condition capable of infecting, having, of course, been infected at some previous date in the "fly country." It may be repeated, that these flies were caught in a most highly infected district, so that if *Glossina morsitans* can remain infective for 50 or 60 days, 1 at least of the 85 ought to have been in the condition which made it capable of conveying the disease.

This development of the trypanosomes in the fly is strikingly like what occurs in the test-tube with Novy's medium. A thousand tubes are inoculated with *Trypanosoma brucei*: the trypanosomes all appear to die off, but 20 days afterwards a peculiarly resistant individual is found in one tube of the thousand, who has adapted himself to the new enviroment, and soon multiplies into myriads. What it is which enables this particular individual to adapt itself to such altered conditions is unknown. It is the merest speculation to call it a sexual act and pick stout forms as females and slender forms as males.

Again, because this late development of the trypanosomes enables a particular fly to remain infective for 100 days, or even possibly for the remainder of its life, it by no means follows that this is the usual method of infection. The mechanical transference of the disease is proved up to the hilt, and for every case which falls a victim to the rare late-infected fly, a thousand must be infected by direct mechanical transference.

Summary of the Experiment which forms the Subject of this Paper.

Before describing at length the experiment which forms the subject of this paper, we may summarise it as follows:—

1. On March 5, 1909, 60 *Glossinæ palpalis* caught on the lake shore were placed in two cages, 30 in each. The flies were fed on two infected monkeys for 2 days. They were then starved for 72 hours to get rid of mechanical transference. The following 5 days they were placed on a healthy monkey, and every successive period of 5 days, or thereabouts, on a fresh monkey, up to 86 days, when the experiment came to an end. The result was, that the first two monkeys remained healthy, but that all the following monkeys, up to 75 days, became infected with *Trypanosoma gambiense*.

2. If 7 days be deducted for the incubation period, then the flies first became infected 18 days after their first feed on an infected animal.

3. There is some evidence that among the 60 flies only 1 was infective. Fifty-four days after the beginning of the experiment each cage was placed on a separate monkey. Up to that time both the cages of flies had been fed on the same animal. Cage A contained, after 54 days, 11 flies. Cage B, 4 flies. Cage A continued to infect monkeys for 21 days more, making a total of 75 days. Cage B did not infect. Again, as was natural, the flies gradually died off during the experiment, and as each fly died it was carefully dissected and examined for trypanosomes. Not a single trypanosome of any kind whatever was seen in any dissected fly up to 75 days, when a fly died in Cage A which was found to be swarming with trypanosomes similar to *Trypanosoma gambiense*. After the death of this fly, Cage A ceased to be infective, and when the experiment was stopped the remaining flies were killed off and dissected, but among them not a sign of a trypanosome could be seen. In the same way the flies remaining in the non-infective Cage B were examined, with a similar negative result.

4. Here follows an interesting and unique observation. A tiny drop of fluid taken from the gut of the 75-day fly injected under the skin of a monkey gave rise to Sleeping Sickness after an incubation period of eight days. This, so far as we are aware, is the first time this has been recorded.

5. It will be seen from the detailed experiment that the flies were starved for three days between several of the experiments. This, of course, was to get rid of the fallacy of mechanical transference.

6. It may be said that perhaps these monkeys became infected by some other means than the flies in the cage—for example, by other biting flies, or by contact. To this it may be answered that there are more than 200

monkeys under observation here, sick and healthy. They are all examined twice a week, but during the last eight months not a single case of accidental infection has taken place.

Details of the Experiment.

Experiment 663.

To ascertain if development of *Trypanosoma gambiense* takes place in the interior of *Glossina palpalis*, and if so, how long does the fly remain infective.

March 5, 1909.—Two batches of *Glossina palpalis* caught on the Lake shore, consisting of 30 flies in each batch, were fed on monkeys, Experiments 568 and 214, whose blood contained numbers of *Trypanosoma gambiense*.

March 6.—The flies again fed as on the 5th, to ensure that as many as possible should get a feed of the infected blood. Nearly all the flies fed on one or other occasion. The flies are kept in a moist atmosphere at 22° C.

The following table gives the principal details of the experiment:—

Table II.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Mar. 5	—	Flies fed on infected monkey			
6	1	„ „ „			
7	2	Flies starved 72 hours			
8	3	„ „			
9	4				
10	5				
11	6	Fed on Monkey 579		−	
12	7				
13	8				
14	9				
15	10				
16	11	„ „ 651		−	
17	12				
18	13				
19	14				
20	15				
21	16	„ „ 652	+		
22	17				
23	18				
24	19				
25	20				
26	21	„ „ 653	+		
27	22				
28	23				
29	24				
30	25				
31	26	„ „ 654	+		
Apr. 1	27				
2	28				

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Apr. 3	29				
4	30				
5	31	Fed on Monkey 655	+		
6	32				
7	33				
8	34				
9	35				
10	36	„ „ 672	+		
11	37				
12	38				
13	39				
14	40	„ „ 722	+		
15	41				
16	42	Starved for 72 hours			
17	43				
18	44				
19	45				
20	46				
21	47				
22	48	Fed on Monkey 727	+		
23	49				
24	50				
25	51				
26	52	Starved for 76 hours			
27	53				
28	54	Cage A fed on Monkey 735 ...	+		
29	55				
30	56				
May 1	57				
2	58	„ B „ „ 736 ...		−	
3	59	Starved for 74 hours			
4	60				
5	61	Cage A fed on Monkey 749 ...	+		
6	62				
7	63				
8	64				
9	65	„ B „ „ 748 ...		−	
10	66	Starved for 72 hours			
11	67				
12	68	Cage A fed on Monkey 765 ...	+		
13	69				May 13.—Flies remaining in Cage B killed and dissected.
14	70				
15	71				
16	72	„ B „ „ 764 ...		−	
17	73	Starved for 72 hours			
18	74				
19	75				May 19. — Fly 866 found dead in Cage A and dissected. Did not feed on Monkey 848.
20	76				
21	77	Cage A fed on Monkey 848 ...		−	
22	78				
23	79				
24	80	Starved for 72 hours			
25	81				
26	82				
27	83				
28	84	Cage A fed on Monkey 911 ...		−	Expts. 848 and 911 healthy on 7th June, 1909.
29	85				
30	86				
31	87	Experiment stopped.			Remaining flies killed and dissected.

Remarks on the Experiment.

Everyone will agree that this is a most interesting experiment. It is evident that a single infected fly did all the mischief, and by good luck this fly was detected. Captain A. E. Hamerton, D.S.O., had charge of the experiment at first, and on his leaving Mpumu about the beginning of May, it fell to Sergeant A. Gibbons, Royal Army Medical Corps. Both are to be congratulated on the results, which are the outcome of care and thoroughness. Captain F. P. Mackie had the good fortune to dissect the fly which did the injury, and which will be fully described later.

INCUBATION PERIOD.

From the experiment may be drawn the incubation period in monkeys bitten by a late-infected fly.

It is remarkable how regular this is in those monkeys which gave a positive result. This shows how very infective Fly 866 was. Apparently each time it bit it infected.

The following table gives the period of incubation in each case:—

Table III.

Date.	Experiment.	Flies first fed.	Trypanosomes appeared in blood.	Number of days before trypanosomes appeared in blood.
1909.		1909.	1909.	
March 19	652	March 19	March 30	11
„ 24	653	„ 24	April 2	9
„ 29	654	„ 29	„ 6	8
April 3	655	April 3	„ 13	10
„ 8	672	„ 8	„ 15	7
„ 13	722	„ 13	„ 20	7
„ 18	727	„ 18	„ 24	6
„ 28	735	„ 28	May 5	7
May 5	749	May 5	„ 11	6
„ 12	765	„ 12	„ 17	5

Leaving out the first experiment, 652, as it is doubtful as to the exact day Fly 866 became infective, this gives an average incubation period of seven days. It would therefore appear that Fly 866 probably infected each animal on the first day it bit it, showing how dangerous such an infected fly is.

DESCRIPTION OF THE *Glossina palpalis*, FLY 866, WHICH WAS DISSECTED 75 DAYS AFTER HAVING FED ON A MONKEY WHOSE BLOOD CONTAINED *Trypanosoma gambiense*.

Experiment 866.

May 19, 1909.—Dissected a *Glossina palpalis*, which was found dead to-day in Cage A of Experiment 663. On removing the viscera by the usual method, the mid-gut was seen to be of a pale salmon-pink. A small quantity of its contents, examined in the fresh condition, was found to contain enormous numbers of trypanosomes. The tube of this part of the intestine was absolutely crammed with active, seething masses of these flagellates. In regard to the other parts of the fly, nothing was seen in the proboscis. In the proventriculus one trypanosome only was found. The salivary glands contained large numbers of altered-looking trypanosomes, the fore-gut many large stout forms, with bright granules. The crop was empty and showed nothing. The Malpighian tubules, hind-gut, and proctodæum also were drawn blank.

In addition to examining these organs in the fresh condition, smears were made and stained. The examination of these stained specimens gave the following results:—

The salivary glands.—These had been carefully removed before the intestine was opened, and therefore had no chance of being fouled. As will be seen from the coloured drawing (Plate 10, fig. 1), the trypanosomes found in these glands differed from those seen in the intestine. The bodies are very irregular in shape, and contain, besides a reddish-stained nucleus, dark deeply-stained coarse chromatin granules. The other cell contents remain unstained. Free chromatin granules and flagella are to be seen scattered over the field. Sometimes the bodies are definitely pear-shaped, with a flagellum coming from the narrow end, and rarely a more definite trypanosome shape can b seen; but never a true trypanosome.

[It is a matter of deep regret that an inoculation experiment was not made with an emulsion of part of the salivary glands.]

The fore-gut.—The fore-gut contained many trypanosomes. The cytoplasm stains a pale blue, and the nucleus a reddish-purple. The micronucleus is not distinctly seen in some of the trypanosomes, but when it is, it is always distinctly posterior to the nucleus. The protoplasm contains many coarse darkly-stained chromatin granules. The undulating membrane is less marked than in the normal blood trypanosome, and the flagellum, which usually springs from a micronucleus-like body, is less deeply stained (Plate 11, figs. 6—13).

The mid-gut.—The mid-gut contained innumerable trypanosomes of the *gambiense* type. Some are dividing, and all have a well-marked nucleus and micronucleus, the latter at or near the posterior extremity. The protoplasm contains many chromatin granules, and an undulating membrane and flagellum are present (Plate 10, figs. 6—16). Many groups, or rosettes, composed of 15 to 20 individuals, occur, the flagella pointing outwards (Plate 11, fig. 1).

The *proboscis, proventriculus, thoracic gut, crop, hind-gut,* and *Malpighian tubes* contained no trypanosomes.

The most interesting thing in this description of the examination of Fly 866 is the condition of the salivary glands. How these trypanosome-like bodies, or derivatives of trypanosomes, got into them is a mystery, and we will content ourselves at present with merely placing the bare fact on record until the salivary glands of similarly infected flies are examined.

There is one fallacy which might be pointed out. It is assumed that Fly 866 became infected on the first or second day of the experiment. It is possible that it became infected when feeding on the fifth day on an animal which showed trypanosomes in its blood a day or two later. This, however, is unlikely, as no other fly showed trypanosomes on dissection.

In order to make the story more complete, on Plate 10, figs. 1—5, is represented the *Trypanosoma gambiense* from the blood of one of the monkeys on which the flies were fed at the beginning of the experiment, and on Plate 11, figs. 2—5, are shown *Trypanosoma gambiense* from the monkey which became infected from the contents of the mid-gut of Fly 866.

Proportion of Infected Flies to Non-Infected in Nature.

In the experiment under consideration it is seen that, in artificially-infected flies, only 1 in 60 showed the phenomenon of late infectivity. In nature the proportion must be less, as many of the flies, in many places at least, can never have fed on an animal whose blood contained *Trypanosoma gambiense.*

That there can be but few under natural conditions Table IV shows. The table is made by subtracting the flies fed on the animal during the last seven days, before trypanosomes were found in the blood, this being the incubation period, from the total number. The experiments consist in catching tsetse flies in the infected area, bringing them to the laboratory and placing them straightway on healthy animals.

The first two experiments were made with *Trypanosoma brucei* and *Glossina morsitans,* and it would appear from them that 104 and 108 flies

Table IV.—Table to show Probable Number of Naturally infected Flies per thousand.

Expt.	Place.	Observer.	No. of flies fed before infection took place.	Result.		Probable No. of naturally infected flies per thousand.
				Positive.	Negative.	
		Trypanosoma brucei—Glossina morsitans.				
225	Zululand	Bruce	104	+		9·6
236	,,	,,	108	+		9·2
		Trypanosoma gambiense—Glossina palpalis.				
94	Uganda	Bruce and Nabarro	89	+		11·2
130	,,	Bruce, Nabarro, and Greig	850	+		1·2
131	,,	,, ,,	506	+		1·9
136	,,	Nabarro and Greig	723		—	
228	,,	Greig and Gray	866	+		1·2
301	,,	,, ,,	2299		—	
45	Leopoldville	Dutton, Todd, and Hannington	457		—	
46	,,	,, ,,	552		—	
128A	River	,, ,,	25		—	
139	,,	,, ,,	262		—	
141	,,	,, ,,	52		—	
182	Kasongo	,, ,,	211		—	
198	,,	,, ,,	2659	+		0·4
203	,,	,, ,,	1789		—	
213	,,	,, ,,	717		—	
52	Uganda	Bruce, Hamerton, Bateman, and Mackie	41		—	
214	,,	,, ,,	3284	+		0·3
568	,,	,, ,,	178	+		5·6
571	,,	,, ,,	850	+		1·2
53*	,,	,, ,,	21		—	
612	,,	,, ,,	615	+		1·6
674	,,	,, ,,	2315	+		0·4

* Animal died.

were used respectively before an infective one was found. This perhaps explains why Bruce's 85 flies failed to infect.

In the experiments with *Trypanosoma gambiense* and *Glossina palpalis* the average is 2·5 per thousand. It is, of course, impossible to tell how many of these positive experiments were infected by mechanical transference or by a late-infective fly; but, in any case, the proportion is small. If this were not so, all the native population of the Lake shore, and most of the Europeans in Uganda, would long ago have been blotted out.

DESCRIPTION OF PLATES.

PLATE 10.

Smear preparation of salivary glands of *Glossina palpalis*, Experiment 866, stained Giemsa, showing irregularly shaped trypanosomes, with unstained protoplasm, reddish-coloured nuclei, and deeply stained chromatin granules. Note the chromatin granules scattered singly about the field, each surrounded by a pale area, fig. 1. × 2000.

Normal *Trypanosoma gambiense* from monkey, Experiment 568, on which the flies were fed at the beginning of the experiment, figs. 2, 3, 4, and 5. × 2000.

Trypanosomes from the mid-gut of infected fly, Experiment 866, figs. 6—16. × 2000.

PLATE 11.

Rosette form from the mid-gut, fig. 1. × 2000.

Trypanosoma gambiense from the blood of monkey, Experiment 868, into which a tiny drop of the contents of the mid-gut of Fly 866 had been injected, figs. 2—5. × 2000.

Trypanosomes from the fore-gut of Fly 866, stained Giemsa, figs. 6—13. × 2000.

A Note on the Occurrence of a Trypanosome in the African Elephant.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908.)

(Received July 5, 1909.)

[PLATE 12.]

As trypanosomes have never been reported as having been observed in the blood of the African Elephant, the Commission thought it would be of interest to note this observation.

In Laveran and Mesnil's book on trypanosomes, translated by Nabarro, on p. 261 it is stated that "the occurrence of Surra (*Trypanosoma evansi*) in elephants in India and Burmah is practically proved. In this connection we have only the statement of G. H. Evans that, in 1893, 14 out of 32 elephants died of the disease in Burmah." The year 1893 is almost prehistoric for trypanosomes. At that time observers had even failed to distinguish between the common rat trypanosome—*Trypanosoma lewisi*—and that of Surra. It may well be, then, that Evans was mistaken in his diagnosis of the species causing this large mortality in elephants.

The African elephant, in whose blood this trypanosome was found, was


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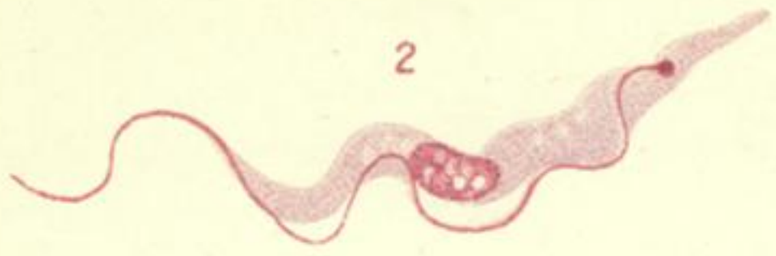
M. E. Bruce del. West, Newman chr.



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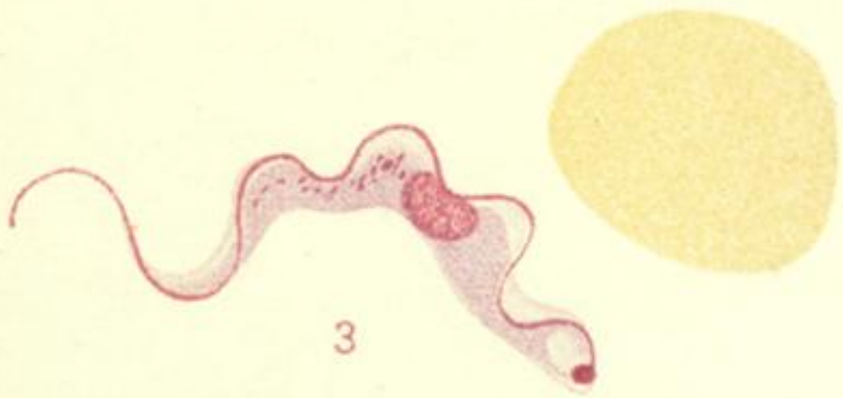

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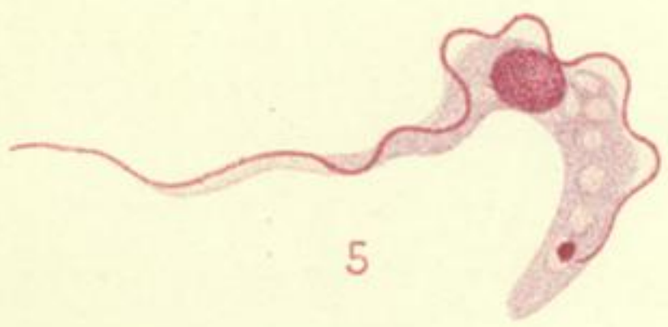

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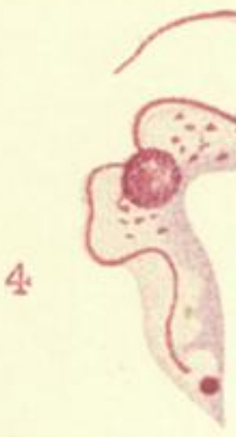

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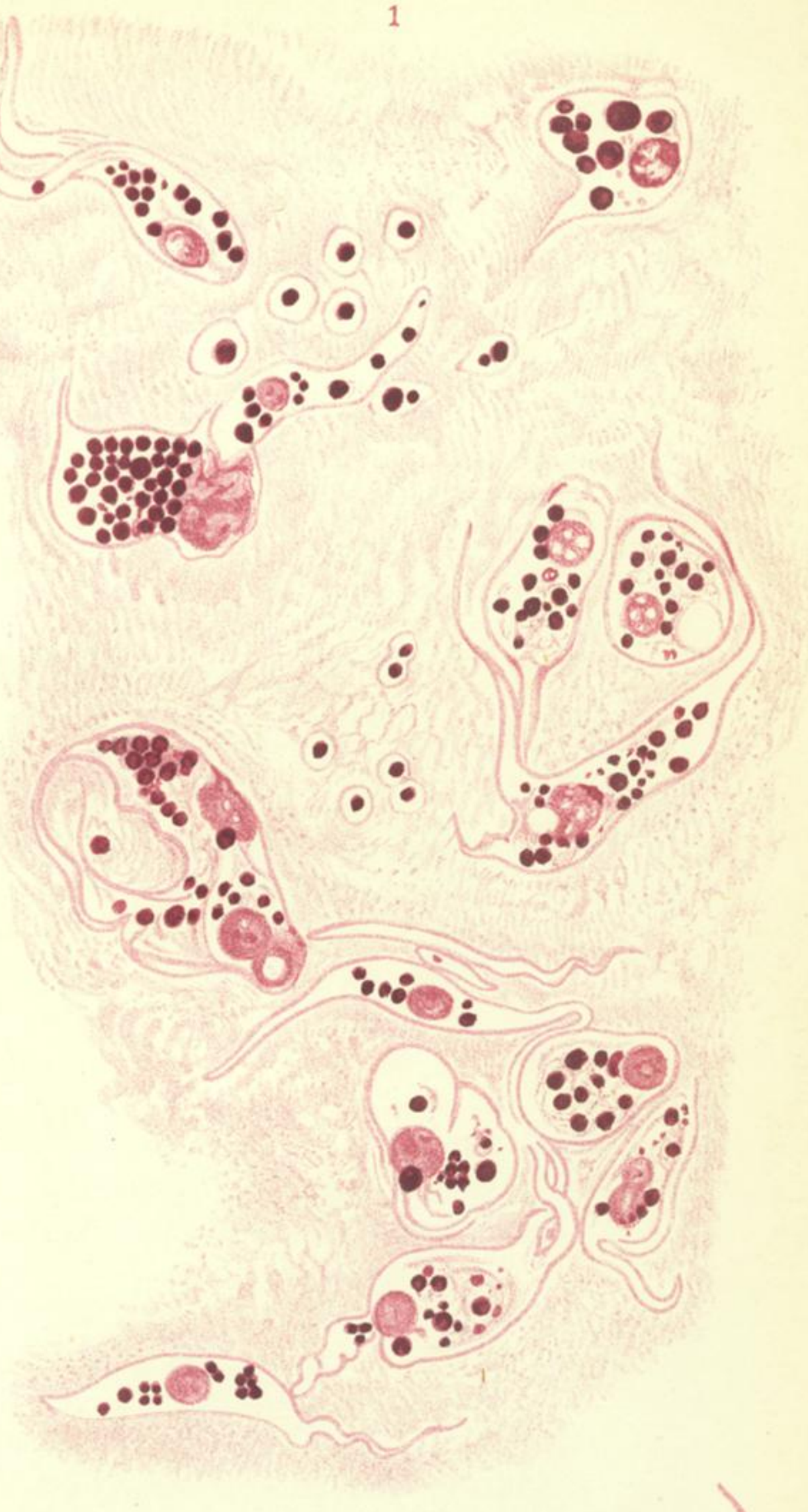

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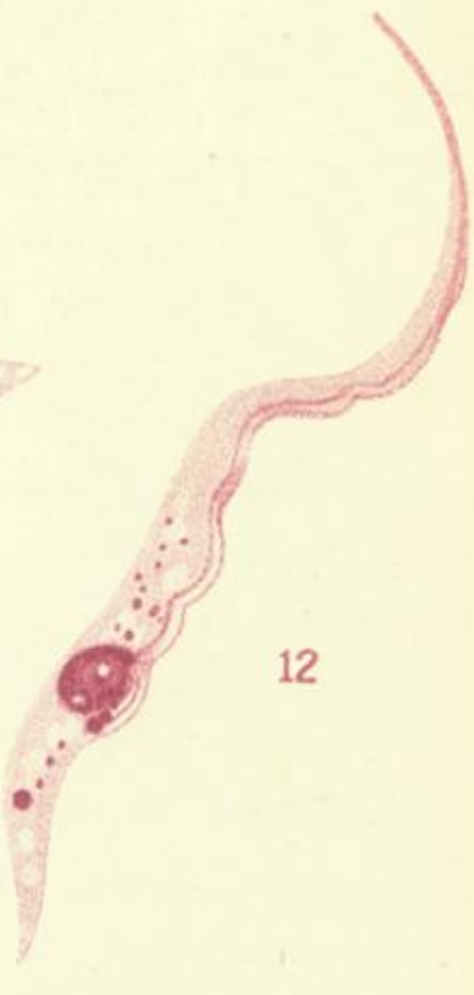

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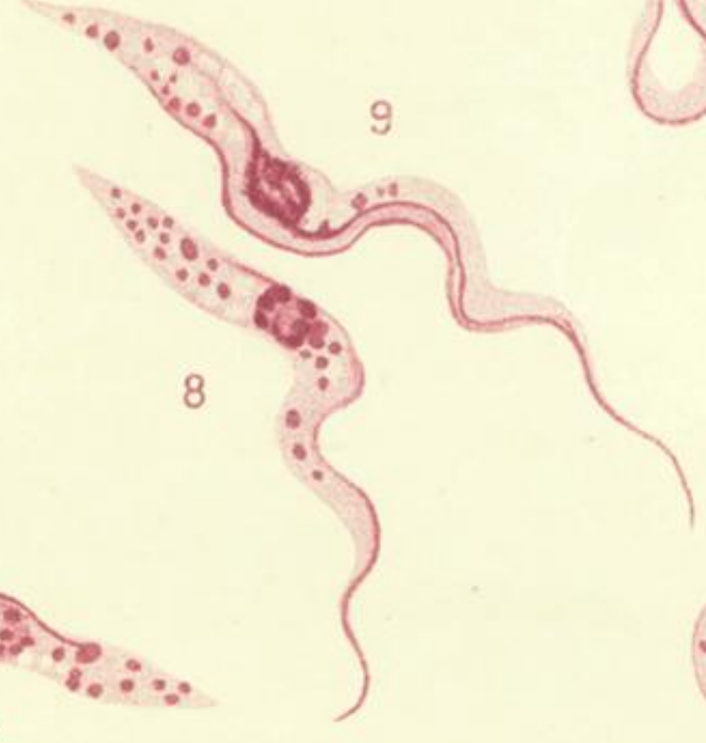

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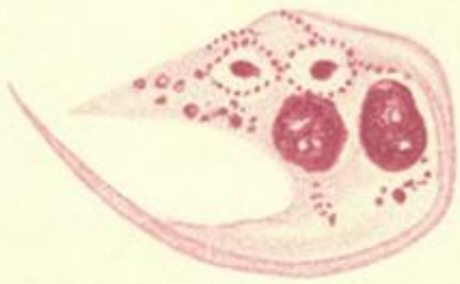

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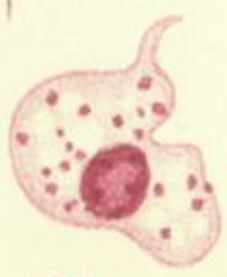

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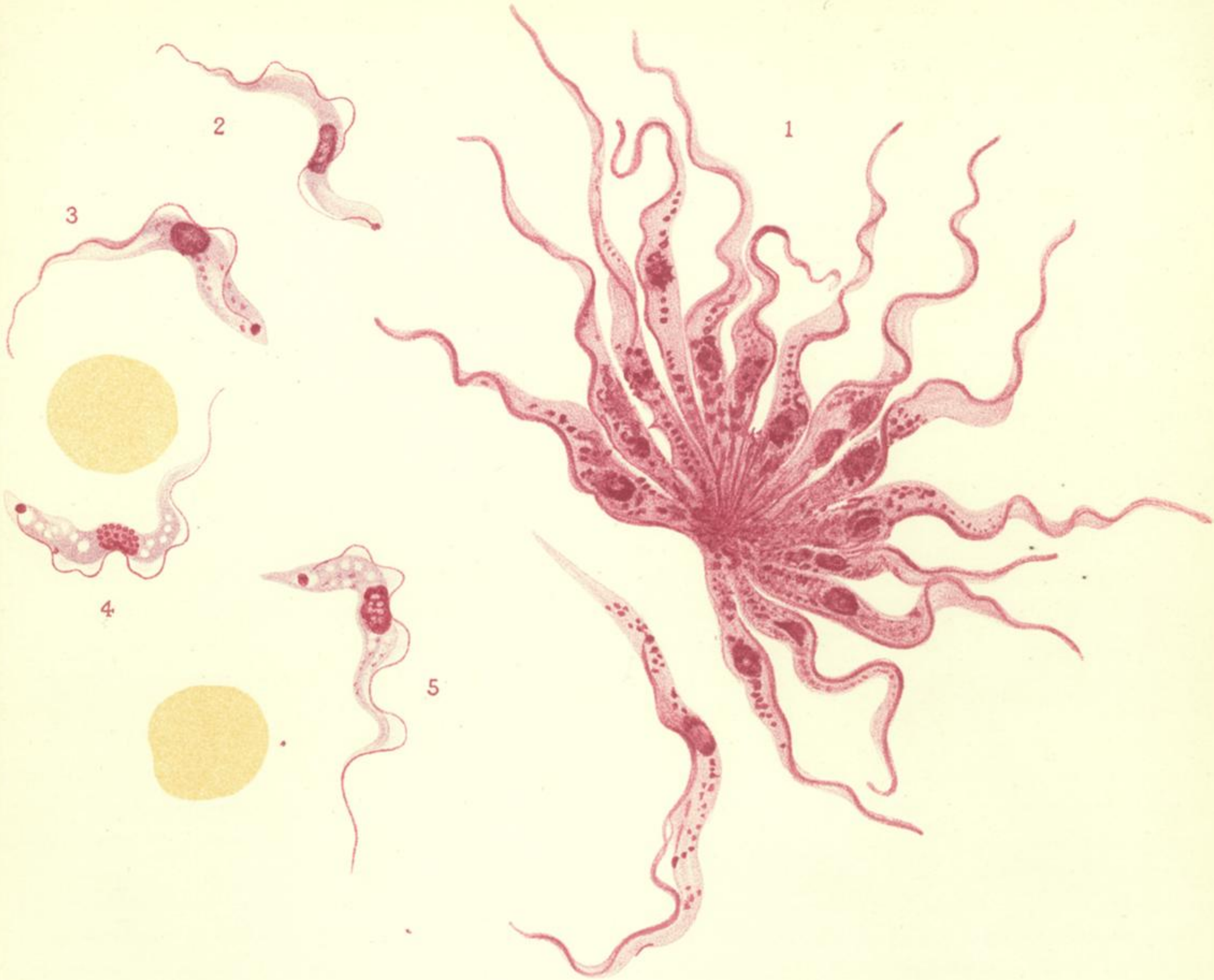
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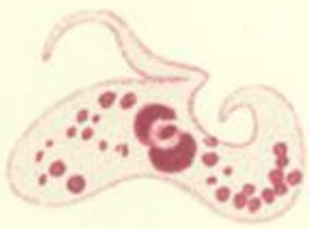
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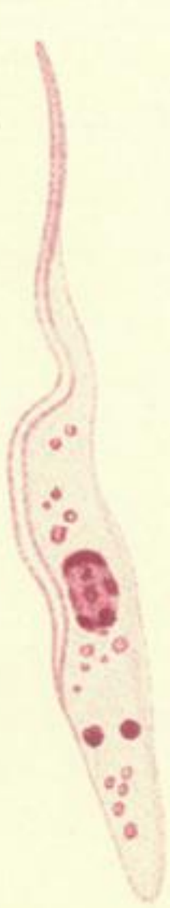
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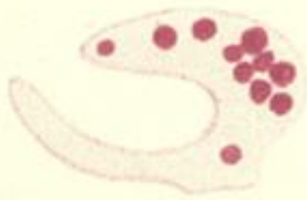
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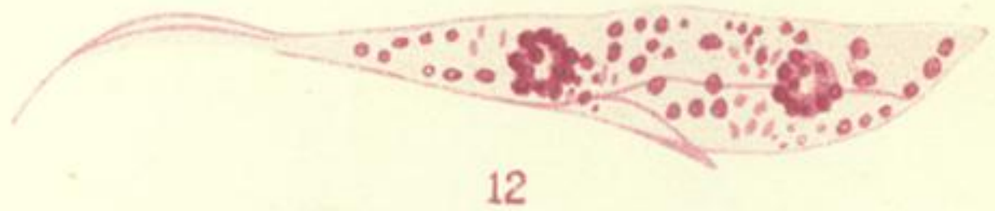
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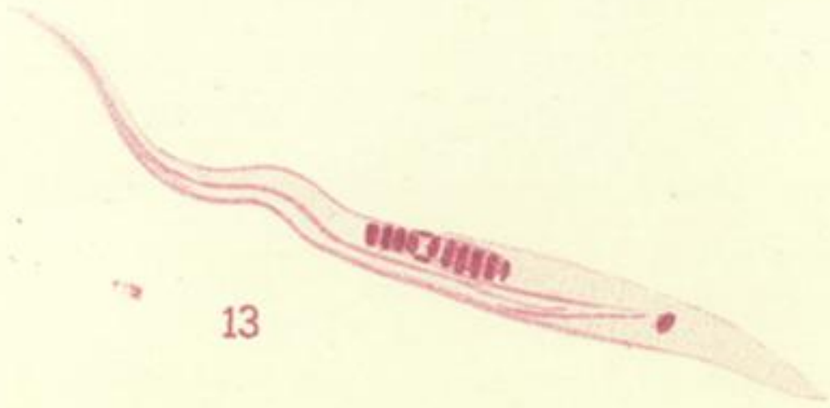
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